BIOLOGY OF THE SOIL-ROOT INTERFACE

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Summary

A short account is given of research on subjects relevant to the understanding of the biology of the soil-root interface, in which recent developments have occurred.

Introduction

Since this subject was reviewed by Rovira and Davey (1974) there has been a considerable change of emphasis in studies of the rhizosphere on the soil-root interface and Bowen and Rovira (1976) drew attention to some of these changes from the traditional studies of the numbers and types of micro-organisms and the characterization of organic materials released from roots into solution under axenic conditions. This paper is not a comprehensive review and deals with areas of research which reflect new thinking of the biology of rhizosphere and the relevance of this approach to plants in the field.

These areas of research in the biology of the soil-root interface are:

(a) Release of organic materials from roots
(b) Microbial colonization of roots
(c) Application of light microscopy to the root surface
(d) Application of electron microscopy
(e) Modelling of the populations in the rhizosphere

(f) Root-associated nitrogen fixation
(g) Mycorrhizas
(h) Interactions between root pathogens and root function

Release of Organic Materials from Roots

For many years estimates of the organic compounds released from roots were based upon results with plants grown under axenic conditions in nutrient solution into which 0.1 to 0.4% of the photosynthate was "exuded" (Rovira and McDougall, 1965). Subsequent studies by Barber and Gunn (1974) showed that roots growing in glass ballotini released from the roots sugars and amino acids equivalent to 5 to 9% of the total weight.

Such a finding brought a new dimension in rhizosphere studies and was supported by Barber and Martin (1976), Martin (1977) and Sauerbeck and Johnen (1977) all of whom reported that carbon released from roots growing in soil amounted to some 20% of the total plant dry matter. Barber and Martin (1976) found that micro-organisms increased the percentage of photosynthetically fixed carbon released into soil by roots from 5-10% under axenic conditions to 12 to 18%, most of the added carbon released as CO_2. Martin (1977) reported that up to 39% of the carbon translocated to the roots was released into the soil and he attributed this to the lysis of root cell walls rather than actual exudation through intact cell walls. This was supported by the E.M. studies of Foster and Rovira (1976) which showed considerable penetration by bacteria of the epidermal and cortical cells in field-grown wheat at flowering.

Microbial Colonization of Roots

To understand the dynamics of colonization of roots by micro-organisms there has been a departure from the plate counting methods by which workers estimated the R:S ratio.

(viz. ratio of the population in the rhizosphere to that in the soil), to the use of model systems in which growth rates on or around the roots are measured. Bowen and Rovira (1976) stressed the need to consider the "generation time" of bacteria on roots as the basis for studying the dynamics of colonization. Barber and Lynch (1977) found that with barley plants growing in solution culture the "doubling time" for the mixed species of bacteria was 23.9, 29.7 and 103.4 hours for the growth periods 0-4, 4-7 and 7-16 days respectively.

Bowen and Foster (1977) eliminated the problem of different colonization times on parts of roots of different ages by planting axenically-grown roots into soil and counting the bacteria on selective media at intervals. They found that pseudomonads had a generation time on roots of 5.2 h compared with 39h for *Bacillus* spp. while in soil *Pseudomonas* spp. had a generation time of 77 h and *Bacillus* spp. over 100 h. Bowen and Foster noted large differences in generation time between species of *Pseudomonas*. This concept of "generation" or "doubling" time on roots or in the rhizosphere is considered the best way to characterize the colonizing ability of different organisms and is an approach which will help establish the basic principles behind the colonization of roots by micro-organisms e.g. growth rates, prior occupation of sites, competition for nutrients, antagonism, etc.

Application of Light Microscopy to the Root Surface

Direct light microscopy of the micro-organisms on roots has shown that only 5 to 10% of the root surface is covered by micro-organisms (Bowen and Theodorou, 1973; Rovira *et al.*, 1976). These studies, in which sound quantitative techniques have been used to assess the populations densities, should dispel the myth that roots were covered by a mantle of micro-organisms - such a myth comes from the astronomical counts expressed per gramme of root

or soil. The application of pattern analysis to the microbial microflora on roots has shown an irregular distribution with the bacteria aggregating on two scales viz. - small clumps and also larger aggregations (Newman and Bowen, 1974). Other work has shown that aggregation is associated with cell junctions (Rovira, 1956; Malajczuk and Bowen, unpublished) and with moribund epidermal cells (Bowen and Foster, 1977).

Fluorescence microscopy using either fluorochromes on roots (Schippers and van Vuurde, 1977) or fluorescent antisera on slide impressions of roots colonized by different strains of *Rhizobium* (Trinick, 1970) has revealed the distributions of bacteria and the competition for colonization between strains of *Rhizobium*.

Application of Electron Microscopy

Our concept of the spatial distribution of the various components of the soil/root interface has been helped greatly by the application of both transmission and scanning electron microscopy. (T.E.M. - Dart and Mercer, 1964; Greaves and Darbyshire, 1972; Old and Nicolson, 1975, 1977; Foster and Rovira, 1976, 1977; S.E.M. - Dart (1971); Campbell and Rovira, 1973; Rovira and Campbell, 1974, 1975.)

Scanning electron micrographs in Figures 1 and 2 show the surface of roots of wheat (1) a healthy root with mucilage spreading over the surface enveloping bacteria and clay particles, (2) a root infected with Take-all fungus in which the mucigel is no longer present and the root is heavily colonized with bacteria. The T.E.M. view of a clover root in Figure 3 shows the dense packing of bacteria for 10 to 15 μm between the root and soil with many types of bacteria, some forming small colonies. The bacteria some distance from the soil-root interface occur either singly or as pairs completely enclosed in clay by comparison with the formation of colonies near the root.

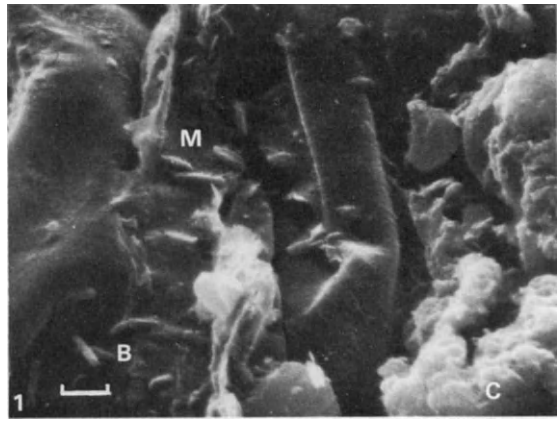


Fig. 1. Scanning electron micrograph of wheat root, M = Mucigel, B = Bacteria, C = Clay. Bar = 5 µm.

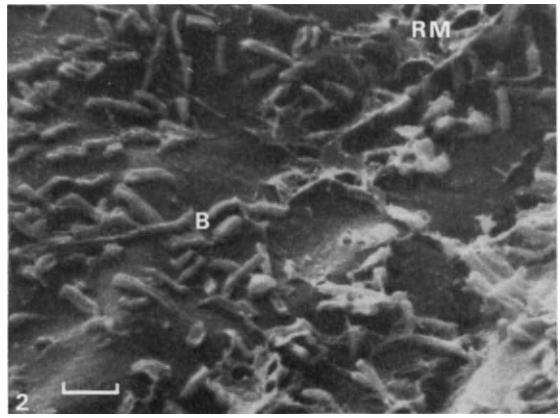


Fig. 2. Scanning electron micrograph of wheat root infected with Take all fungus. B = Bacteria, RM = Remnants of mucigel. Bar = 5 µm.

The rhizosphere of *Paspalum* grown in permanent pasture under irrigation shows a thick layer of mucigel being dissolved by *Spirillium*-like bacteria then a sharp boundary to the clay-organic matter material 10 to 30 µm from the root; the colony of bacteria developed in this clay-organic material was most probably nourished by soluble material from the root (Fig. 4).

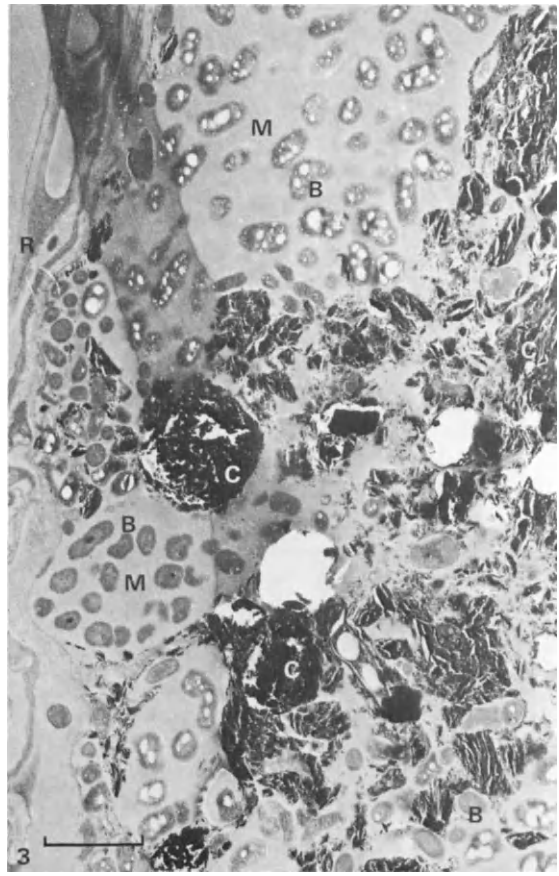


Fig. 3. Transmission electron micrograph of root of subterranean clover (*Tr. subterraneum*). R = Root, B = Bacteria, C = Clay, M = Mucilage - probably bacterial in origin. Bar = 5 µm.

Modelling of the Populations in the Rhizosphere

The quantification of the energy material lost by roots into soil and of the microbial population on roots led Bowen and Rovira (1973) to ask "Are modelling approaches useful in rhizosphere biology?". They concluded that there was then still insufficient information to produce such a model. However, in 1977, Newman and Watson developed a computer model to predict the effects of changes in soil water, root density, root exudation, initial microbial

populations and in response of micro-organisms to substrate on the concentrations of substrates and micro-organisms at different distances from the root. Their prediction of a sharp reduction of the population away from the root surface has been supported by the T.E.M. studies. The modelling concept will be useful to predict the changes in microbial populations with changes in conditions at the soil-root interface and will help workers ask critical questions to guide future research.

Fig. 4. Transmission electron micrograph of root of *Paspalum notatum*. R = Root, M = Mucigel, S = *Spirillum*-like bacterium, C = Clay, O.M. = Organic Matter, B.C. = Bacterial Colony. Bar = 5 μm.

Root-associated Nitrogen Fixation

Reports of agronomically significant amounts of nitrogen being fixed in the rhizosphere of grasses in the cooler regions of Western Australia came from Parker (1957) and later from Dobereiner and Campelo (1971) with the association between the roots of *Paspalum* and *Azotobacter paspali*. Later reports that linked nitrogen fixation under tropical grasses with photosynthesis (Dobereiner and Day, 1975) and associated the fixation with *Azospirillum (Spirillum lipoforum)* living inside the cortical cells of the roots (Dobereiner, 1977) indicate that significant amounts of nitrogen are fixed when the soil is at high temperature and wet. The manipulation of this system for agronomic benefit may be more difficult than managing the legume-*Rhizobium* symbiosis but considerable research is being undertaken in the hope that such fixed nitrogen may replace fertilizer nitrogen.

Mycorrhizas

The biology and function of the ectomycorrhizal fungi have been understood for a long time (Marks and Kozlowski, 1973) but the appreciation of the importance of the vesicular-arbuscular mycorrhizas (VAM) is a recent phenomenon. The tremendous growth responses of plants in soils low in phosphate (Mosse, 1973; Tinker, 1975) and the improvement in nitrogen fixation by legumes when infected with V/A mycorrhiza (Crush, 1974; Daft and El-Gaimi, 1975) has aroused interest in countries with extensive areas of phosphate-deficient soil. This is an important area of research in the biology of the soil-root interface and, like the root-associated nitrogen fixation, justifies more research with agronomic aims (Bowen and Bevege, 1976).

Interactions between Root Pathogens and Root Function

The presence of root pathogens in the soil-root interface may be more common than their absence. Hence it is relevant to present some results on the effects of pathogens on root function.

One of the major root pathogens of cereals in Southern Australia is cereal cyst nematode (*Heterodera avenae*) (Meagher, 1972; Rovira, 1977). This pathogen affects the shoot to root ratio, response of plant to phosphate (Table 1), response of root growth to soil temperature (Fig. 5) and results in the formation of thick layers of mucigel colonized by fungi and bacteria (Figs. 6 and 7).

TABLE 1

Interactions between cereal cyst nematode (H.avenae), superphosphate and growth of wheat*

Nematicide**	Superphosphate	Seminal Roots/Plant		Tops/Plant		Ratio:- Top Wt. / Root Wt.
		Length (cm)	Dry Wt. (mg)	Leaf area (cm^2)	Dry Wt. (mg)	
-	-	434	63	7	37	0.59
+	-	859	60	16	103	1.70
-	+	664(53)#	76(19)	13(94)	73(84)	0.96
+	+	892(4)	59(0)	19(19)	119(16)	2.02

* 580 eggs/100 g soil

** Aldicarb (Temik, Union Carbide) for complete control at 9 mg a.i./kg soil (equiv. 9 kg a.i./ha)

\# ( ) Percent increase with superphosphate at 200 mg/kg

Pot experiment: Soil kept moist at 15°C for 4 weeks before seeding to stimulate hatching of nematodes, wheat grown at a root temperature of 10°C for 4 weeks. Values are the means per plant calculated from five replicate pots with 5 plants/pot containing 400 g soil.

The losses of cereal yields due to infection of roots by Take-all fungus (*Gauemannomyces graminis*) make it second in importance to stem rust on a world-wide basis. One of its symptoms is the premature ripening or "haying-off" of crops, and we have shown that infection of roots of wheat at both the seedling and flowering stages in-

creases the water potential of leaves and the osmotic potential of the sap (Table 2). These changes might be due to the effects of the *G.graminis* on the translocation of assimilate from leaves to roots (Clarkson *et al.*, 1975) and the blocking of the xylem vessels by hyphae and gum associated with the hyphae (Fig. 8).

The influence of these two soil-borne root diseases on the mineral composition of the leaves of wheat is shown in Table 3.

The interactions between micro-organisms at the soil-root interface are important in determining the balance of micro-organisms and, in the case of root diseases, the extent of biological control. Rovira and Campbell (1975) demonstrated with S.E.M. that in soils in which Take-all was suppressed the hyphae of *G.graminis* were colonized by rod-shaped bacteria (Fig. 9) and at a later stage the

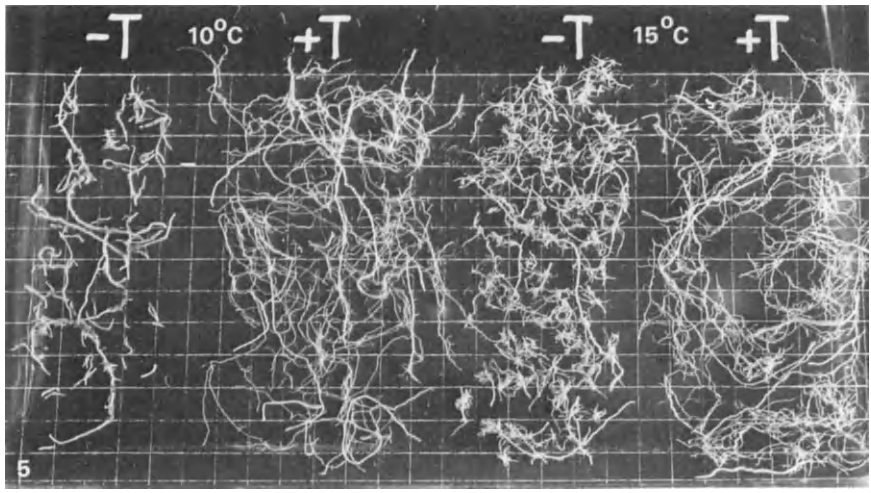
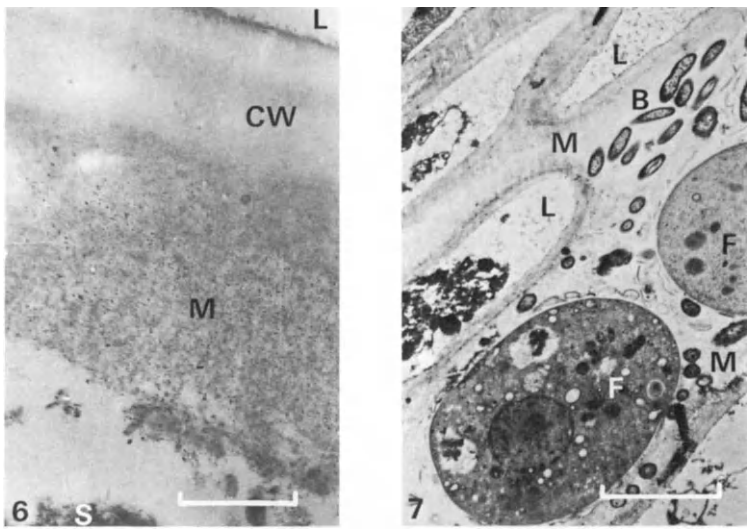


Fig. 5. The effect of soil temperature on growth of wheat roots with (-T) and without (+T) cereal cyst nematode (CCN). The same soil, containing CCN, was incubated at 15°C for 4 weeks to promote hatching, planted to wheat grown in the glasshouse with 10° and 15°C root temperatures for 4 weeks. -T = Superphosphate only. +T = Superphosphate plus the nematicide "Temik". 1 cm grid in background. Cm root/plant at 10°: with CCN 133, without CCN 460. Cm root/plant at 15°: with CCN 400, without CCN 616.



Figs. 6 and 7. Transmission electron micrograph showing the formation of mucilage in and around root infested with CCN. L = Lumen of plant cell, C.W. = Plant cell wall, M = Mucilage, B = Bacteria, F = Fungus, S = Soil. Bar = 5 µm.

TABLE 2

Influence of Take-all fungus (G.graminis) on the osmotic potential of sap and water potential+ of leaves of wheat

Stage of Growth:	Three leaf		Flag leaf at Flowering	
	Healthy	Diseased	Healthy	Diseased
Leaf Water Potential	-554 ± 80	-1286 ± 203	-1640 ± 56	-1640 ± 53
Osmotic Potential	N.M.*	N.M.	-1370 ± 89	-1780 ± 83

± Standard error of the mean
* N.M. = not measured
+ Water potential, kPa

TABLE 3

The effect of cereal cyst nematode (H.avenae) and Take-all fungus (G.graminis) on the mineral content of the tops of wheat

(a) Cereal cyst nematode

Soil Treatment	Top Dry Wt. g/m row	Mineral Content (%)** P	S	K	Ca	Si	Cl	Mg
Nil	3*	0.46	0.55	4.5	0.92	1.6	0.86	0.19
Nil	8*	0.46	0.43	5.1	0.55	1.6	0.97	0.16
Nil	22#	0.45	0.41	6.2	0.28	1.3	1.6	0.16
Nematicide***	32	0.49	0.40	5.8	0.31	1.4	1.6	0.16

(b) Take-all

Disease Rating+	Top Dry Wt. (g/plant)	Mineral Content (%)** P	S	K	Ca	Si
5	0.2	0.44	0.28	2.2	0.28	1.3
4	0.5	0.48	0.35	5.4	0.32	1.3
1 to 3	0.6	0.61	0.43	6.7	0.30	1.6
0	0.8	0.72	0.45	6.9	0.36	2.3

Superphosphate applied at 200 kg/ha.
* Plants severely stunted.
** XRF analyses conducted by Mr. J.T. Hutton on plants sampled 8 weeks after seeding at Turretfield Research Centre.
*** Aldicarb ("Temik" from Union Carbide Ltd.) at 2.4 kg a.i./ha gave complete control.
\# Roots with light infestation.
\+ Disease rating 0 = no disease; 1 to 3 = "light" infection confined to lesions on the seminal roots; 4,5 = "heavy" infection extending from seminal roots to crown.

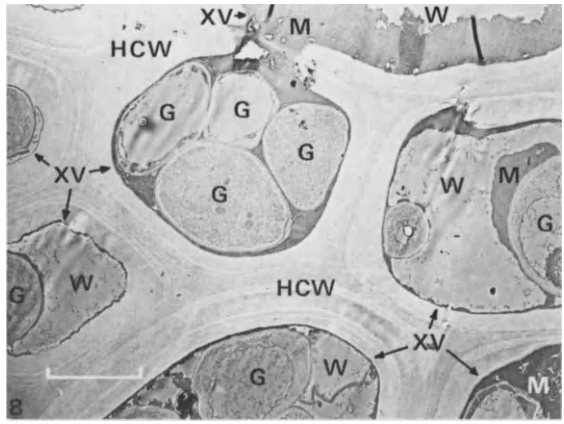


Fig. 8. Micrograph of cross section of wheat root infested with Take-all fungus (*G.graminis*). G = *G.graminis*, M = Mucilage, H.C.W. = Host-cell walls, XV = Xylem vessels, W = Water filled space. Bar = 5 µm.

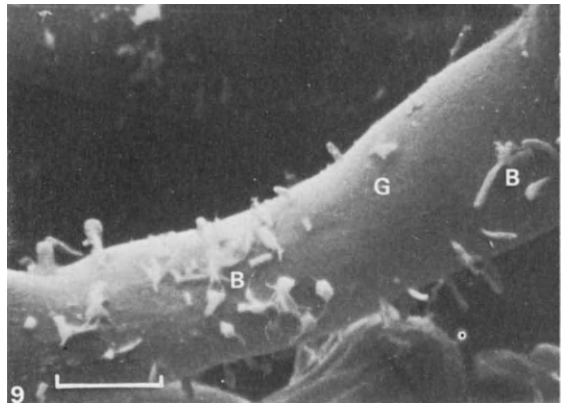


Fig. 9. Scanning electron micrograph of hyphae of *G.graminis* (G) colonized by bacteria (B). Bar = 5 µm.

hyphae collapsed and showed holes most probably caused by the bacteria (Fig. 10).

Conclusions

The biology of the soil-root interface is extremely complex but by the development of new techniques we now have a far better understanding of the release of organic materials from roots, the dynamics of root colonization

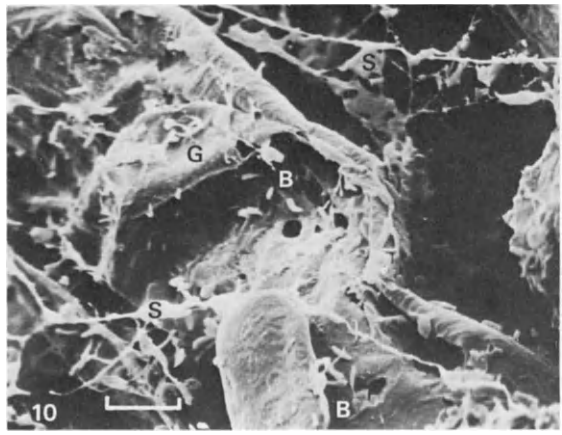


Fig. 10. Scanning electron micrograph of hyphae of *G. graminis* (G) showing the lysis by bacteria (B). Remnants of the hyphae and associated mucilage appear as strands (S). Bar = 5 µm.

and the ultra-structure of the soil-root interface. The application of mathematical models to this zone of soil with validation by experiments will help define the critical questions to be answered.

The ultimate agricultural goal in studies of the biology of the soil-root interface must be the manipulation of micro-organisms in this zone to increase plant growth. Information is required for such manipulation, apart from the Rhizobium-legume association, on endotrophic mycorrhizas, nitrogen fixation with grasses, biological control of root diseases, and the introduction of micro-organisms which stimulate plant growth.

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